

DOCKET NO: 244855US0

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

IN RE APPLICATION OF

:

SUSUMU HIROSE, ET AL.

: EXAMINER: S. KAUSHAL

SERIAL NO: 10/699,852

:

FILED: NOVEMBER 4, 2003

: GROUP ART UNIT: 1633

FOR: METHOD FOR DETECTING NEGATIVELY SUPERCOILED DNA

REMARKS IN SUBMISSION OF DECLARATION

COMMISSIONER FOR PATENTS  
ALEXANDRIA, VIRGINIA 22313

SIR:

Supplemental to the Request for Reconsideration filed on December 6, 2006,  
Applicants submit the executed Rule 132 Declaration referred to at page 3 therein.

As discussed in the previous response, the claimed method provides many benefits to this medical field. For instance, as described in the Rule 132 Declaration submitted herewith, the claimed method makes it possible to discriminate between human leukemia cells and leukocytes.

Application No. 10/699,852

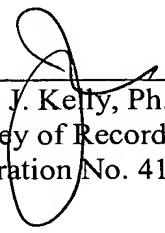
In view of the previous response and the Declaration submitted herewith, Applicants respectfully submit that the present application is in condition for allowance. Early notice to this effect is earnestly solicited.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.  
Norman F. Oblon

Customer Number  
**22850**

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(OSMMN 06/04)



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James J. Kelly, Ph.D.  
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Registration No. 41,504



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IN RE APPLICATION OF :  
SUSUMU HIROSE, ET AL. : EXAMINER: S. KAUSHAL  
SERIAL NO: 10/699,852 :  
FILED: NOVEMBER 4, 2003 : GROUP ART UNIT: 1633  
FOR: METHOD FOR DETECTING :  
NEGATIVELY SUPERCOILED DNA :

DECLARATION UNDER 37 C.F.R. § 1.132

COMMISSIONER FOR PATENTS  
ALEXANDRIA, VA 22313-1450

SIR:

Now comes Susumu Hirose who

deposes and states:

1. That I am a graduate of Graduate Course of the University of Tokyo and received my Ph. D. of Science degree in the year 1971.
2. That I have been employed by National Institute of Genetics for 20 years as a Professor in the field of Molecular Biology.

3. That the following experiments were carried out by me or under my direct supervision and control.

A study to discriminate between human leukemia cells and leukocytes using a biotinylated psoralen derivative was performed as follows.

#### Methods:

A human acute promyelocytic leukemia cell line NB4 was grown in RPMI 1640 medium (GIBCO, NY) supplemented with 10% fetal calf serum at 37°C and 5% CO<sub>2</sub> to 1x10<sup>6</sup> cells/ml. The culture was incubated with saline or 1 µM all trans-retinoic acid (Sigma, MO) for 24 hr and then was treated with 0.2ng/ml of a biotinylated psoralen containing a delivery peptide N-(2,5,9-trimethyl-7-oxo-7H-furo [3,2-g] chromen-3-ylmethyl)-succinyl-YGRKKRRQRRR-K (biotin)-NH<sub>2</sub> for 10 min. The culture was illuminated with a long wave (365nm) UV lamp (UVP model UVL-21) for 10 min to crosslink the psoralen derivative. The cells were washed with saline and fixed with methanol. The biotinylated psoralen was detected with Alexa Fluor 488-labeled streptavidin (Molecular Probes).

#### Results:

In the absence of retinoic acid, strong signals of biotinylated psoralen were detected in the nucleus of a leukemia cell (left panel). However, the signals became faint after induction of differentiation into leukocyte with retinoic acid (right panel).

4. The undersigned petitioner declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

5. Further deponent saith not.

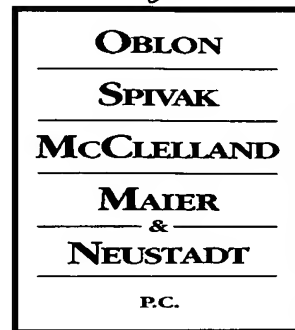
Susanna Harris  
Signature

December 12, 2006  
Date



Docket No.: 244855US0

COMMISSIONER FOR PATENTS  
ALEXANDRIA, VIRGINIA 22313



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RE: Application Serial No.: 10/699,852  
Applicants: Susumu HIROSE, et al.  
Filing Date: November 4, 2003  
For: METHOD FOR DETECTING NEGATIVELY  
      SUPERCQUIRED DNA  
Group Art Unit: 1633  
Examiner: S. KAUSHAL

SIR:

Attached hereto for filing are the following papers:

**Remarks in Submission of Declaration &  
Declaration under 37 C.F.R. §1.132 (EXECUTED)**

Our check in the amount of **\$0.00** is attached covering any required fees. In the event any variance exists between the amount enclosed and the Patent Office charges for filing the above-noted documents, including any fees required under 37 C.F.R. 1.136 for any necessary Extension of Time to make the filing of the attached documents timely, please charge or credit the difference to our Deposit Account No. 15-0030. Further, if these papers are not considered timely filed, then a petition is hereby made under 37 C.F.R. 1.136 for the necessary extension of time. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT, P.C.  
Norman F. Oblon

Customer Number

**22850**

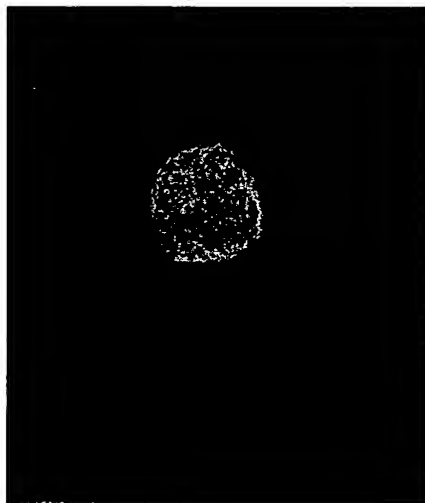
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+retinoic acid  
(under differentiation  
into leukocyte)



-retinoic acid  
(leukemia cell)

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